Chemical Name: Afidopyropen
USEPA PC Code: 026200
USEPA MRID: 49689234
USEPA DP Barcode: 435146
PMRA Data Code: 9.2.4.6
PMRA Study No. (UKID): 2627517
Data Requirement (Guideline): OECD Guidance Doc. No. 75

Test Material: BAS 440 00 I (TEP, VERSYS™) **Purity:** 9.8%

Active Ingredient: Afidopyropen
IUPAC Name: [(3*S*,4*R*,4a*R*,6*S*,6a*S*,12*R*,12a*S*,12b*S*)-3-(cyclopropylcarbonyloxy)-1,2,3,4,4a,5,6,6a,12a,12b-decahydro-6,12-dihydroxy-4,6a,12b-trimethyl-11-oxo-9-(3-pyridyl)-11*H*,12*H*-benzo[*f*]pyrano[4,3-*b*]chromen-4-yl]methylcyclopropane carboxylate
CAS Name: [(3*S*,4*R*,4a*R*,6*S*,6a*S*,12*R*,12a*S*,12b*S*)-3-(cyclopropylcarbonyl)oxy)]-1,3,4,4a,5,6,6a,12,12a,12b-decahydro-6,12-dihydroxy-4,6a,12b-trimethyl-11-oxo-9-(3-pyridyl)-2*H*,11*H*-naphtho[2,1-*b*]pyrano[3,4-*e*]pyran-4-yl]methyl cyclopropanecarboxylate
CAS No.: 915972-17-7
Synonyms: INSCALIS™

Primary Reviewer: Cameron Douglass, Ph.D.
Biologist, USEPA/OCSPP/OPP/EFED/ERBIV
Signature: Cameron Douglass 2018.02.15 15:28:10 -05'00'
Date: 15 February 2018

Secondary Reviewer: Thomas Steeger, Ph.D.
Senior Science Advisor, USEPA/OCSPP/OPP/EFED/ERBIV
Signature: THOMAS STEEGER
Date: 15 February 2018

PMRA Reviewer: Vedad Izadi
Evaluation Officer, PMRA/EAD/ERSII
Date: 13 October 2017

Date Evaluation Completed: 13 October 2017

CITATION: Franke M. 2015. Effects of BAS 440 00 I on the honeybee *Apis mellifera* L. under semi-field conditions (tunnel test) with additional assessments on colony and brood development. BioChem agrar Labor fur biologische und chemische Analytik GmbH, Gerichshain, Germany. Report No. 421110. Sponsor: BASF SE. Report No. BASF Reg. Doc. #: 2016/1000185. USEPA MRID 496892-34. PMRA UKID 2627517.

Executive Summary:
The semi-field (tunnel) study tested the effects of the afidopyropen formulated end-use product BAS 440 00 I (VERSYS™; 9.8% active ingredient) on honeybee (*Apis mellifera*) colonies with the intent of examining brood (*i.e.*, eggs, larvae, pupae) and colony strength (number and condition of adult bees/brood and available food reserves). The study design was based in part on OECD Guidance

Document No. 75. Nucleus bee colonies (containing 7,050 ± 155[1] adult bees/colony) within individual enclosures containing phacelia (*Phacelia tanacetifolia*) in full bloom were exposed to either 0.5 L/ha (50 g a.i./ha; 0.045 lbs a.i./A) of BAS 440 00 I, the insect growth regulator fenoxycarb (300 g a.i./ha), the organophosphate insecticide dimethoate (480 g a.i./ha), or a water (negative) control treatment while bees were actively foraging. The negative control and afidopyropen groups consisted of four replicate tunnels while the fenoxycarb group contained three replicate tunnels and the dimethoate group consisted of a single tunnel. An additional negative control and afidopyropen tunnel were included for monitoring residue levels of afidopyropen and its transformation product M440I007; however, no biological data were collected from these additional tunnels. Across all four groups, each tunnel contained a single nucleus colony; colonies were acclimated to the tunnels four days before applications. Colonies were maintained in the tunnels for 7 days after treatments (DAT, "exposure phase"), and then transferred to a remote monitoring site without a bee-attractive flowering crop for 19 days ("monitoring phase"). Adult and larval/pupal mortality were recorded daily from four days before, to 26 days after, treatments (-4 to 26 DAT); assessments included bee foraging activity (-4 to 7 DAT), colony condition (food stores, brood status), colony strength (numbers of adults and pupae), and brood development indices (brood index, brood compensation index, and brood termination index) at 4, 7, 13, 20 and 26 DAT.

The preliminary brood check indicated healthy colonies with all brood stages present, and a sufficient supply of nectar and pollen. Throughout the study, the number of food or brood cells did not differ statistically among treatment groups. Treatment rates were not confirmed analytically and are therefore based on nominal treatment levels.

There were no statistically significant ($p < 0.05$) differences in adult worker bee mortality between afidopyropen-treated groups and the negative control during the pre-application or monitoring phases of the study; but during the exposure phase, mean adult honey bee mortality in afidopyropen-treated colonies was significantly ($p<0.05$) different (209% higher) compared to negative control tunnels. This increase in adult mortality was largely due to adverse treatment effects on the day of application through 3 DAT, after which worker bee mortality in afidopyropen-treated tunnels was similar to that in control tunnels. There was no mortality of pupae reported in any of the afidopyropen-treated colonies at any point in the study. There were no statistically significant ($p < 0.05$) differences in foraging activity between afidopyropen-treated colonies and the negative control during the pre-application phase of the study, but during the exposure phase of the study, relative to negative control colonies mean foraging activity in afidopyropen-treated tunnels was significantly ($p<0.05$) different (21% lower). Again, this adverse effect was largely due to reduced foraging activity on 0 DAT, and for the remainder of the exposure phase, foraging activity in afidopyropen-treated tunnels was similar to that in control tunnels. The mean number of adult worker bees in afidopyropen-treated colonies was comparable to that in control tunnels throughout the study except at 26 DAT, when the mean number of worker bees in afidopyropen-treated colonies was significantly ($p<0.05$) different (19% lower) than the mean number of worker bees in the negative control tunnels. There were no significant differences in brood strength (*i.e.*, amount of eggs, larvae or pupae) or food stores (*i.e.*, amount of nectar or pollen) in afidopyropen-treated colonies relative to the negative control at any point in the study. Similarly, there were no significant differences in the mean brood index, brood compensation index, or brood termination rate relative to the negative control at any point in the study.

[1] Note that all means in this summary are followed by ± one standard error (SE).

Results Synopsis:
The study is generally consistent with OECD Guidance Document No. 75, although there are some potentially important study deviations and deficiencies. Treatment levels were not analytically verified in the study, and due to possible effects of weather prior to and immediately following treatments, there is some uncertainty regarding actual afidopyropen exposure levels. Additionally, study data from fenoxycarb-treated colonies were highly variable, and so there is additional uncertainty as to how consistent applications of the afidopyropen and fenoxycarb items were across tunnels. However, residue monitoring during the study provides some evidence that bees were appropriately exposed to the afidopyropen.

Honey bee colonies treated with formulated afidopyropen at 50 g a.i./ha (0.04 lbs a.i./A) during active bee flight exhibited significant ($p<0.05$) adverse effects on adult worker bee mortality (209% higher), foraging activity (21% lower), and colony strength (19% lower) resulting in a no-observed adverse effect level (NOAEL) of <50 g a.i./ha under the conditions tested. Adverse treatment effects occurred primarily in the first several days of the exposure phase of the study, after which by almost all measures afidopyropen-treated colonies were roughly similar to negative control colonies.

EPA Classification: Supplemental (should only be used qualitatively)
PMRA Classification: Reliable with restrictions

I. DATA SOURCE

USEPA MRID No.:	49689234
PMRA UKID No.:	2627517
Study Title:	Effects of BAS 440 00 I on the honeybee *Apis mellifera* L. under semi-field conditions (tunnel test) with additional assessments on colony and brood development.
Study Author(s):	Franke M.
Testing Laboratory:	BioChem agrar Labor fur biologische und chemische Analytik GmbH, Gerichshain, Germany.
Laboratory Report No.:	421110
Sponsor Study No.:	BASF Reg. Doc. #: 2016/1000185
Study Completion Date:	17 December 2015
Data Access:	Data submitter is data owner
Data Protection Claimed:	Yes

II. MATERIALS AND METHODS

Test Guideline: OECD Guidance Doc. No. 75 (2007)
Deviations from Guideline:

- The quantities of material applied (*i.e.*, in test solutions) in both the test item (afidopyropen) and the reference items (fenoxycarb and dimethoate) treatments were not verified analytically.
- The acclimation period for honey bee colonies in this study (4 days) is longer than what is recommended (2-3 days) in OECD Guidance Document No. 75; though not explicitly stated by the study author, weather data indicate that it was relatively rainy (and presumably cloudy) for the several days before treatments were made, which could explain the extended acclimation period *(see Reviewer's Comments for additional discussion).*

- At -4 DAT, the mean daily temperature was 13.2 °C; OECD Guidance Document No. 75 notes that daytime temperatures below 15 °C may inhibit honeybee foraging activity.

GLP Compliance: Yes; signed GLP certificate was included and reported no guideline deviations. Laboratory certified by the Staatsministerium fur Umwelt und Landwirtschaft, Freistaat Sachsen.

A. MATERIALS

Test Material: BAS 440 00 I (VERSYS™)

Test Material Identity Batch No. FD-130925-0022; a yellow, liquid formulation comprising afidopyropen (BAS 440 I): 100 g/L (nominal), 98.2 g/L (9.8% measured).

Details on Preparation and Application of Test Materials:
All substances were applied in 400 L/ha water using a calibrated, portable plot sprayer. Applications were made to fully flowering phacelia (BBCH 65).

Analytical Monitoring: None reported.

Details on Analytical Monitoring:
N/A

Reference material I: Insegar™ (fenoxycarb: 250 g/kg [nominal]); batch no: SM02K434; grey solid (water dispersible granules)

Reference material II: Dimethoate™ 400 EC (dimethoate: 400 g/L [nominal]); batch no: FRE-000926; blue liquid (emulsifiable concentration)

Vehicle: None

Test Organism (Species): *Apis mellifera* L. (honeybee)

Animal Group: Arthropoda/Insecta/Hymenoptera/Apidae

Details on Test Organisms: Healthy honeybee colonies, containing eleven combs consisting of 7 - 10 brood combs with all brood stages and sufficient food supply, were used for the study. At the first brood assessment, *i.e*., brood fixation day zero (BFD 0) two days prior to treatment (-2 DAT), colonies contained 6,075-7,988 adult bees. Bees in the colonies were free of clear visual signs of disease or pests, and no unusual occurrences were reported in colonies prior to treatments. Sister queens from 2014 were used to produce colonies which were as uniform as possible (source: BioChem agrar GmBH, Gerichshain, Germany).

B. STUDY DESIGN AND METHODS

Study Type: Semi-field (tunnel) study

Test Duration Type: Long-term toxicity test.

Limit Test: None reported

Total Exposure Duration: 7 d (0 – 7 DAT)

Post-Exposure Observation Phase:
19 d (8 – 26 DAT) for all endpoints

Remarks: Bee mortality was assessed daily beginning four at -4 DAT and ending at 26 DAT. Bee mortality in the tunnels was evaluated using linen sheets

(area approximately 18 m^2) laid at ground level inside the front, middle and back of the tunnels, as well with dead zone dead bee traps at each hive entrance; mortality at the monitoring site was evaluated using only dead zone dead bee traps. Foraging activity of the bees, and overall behavior, were assessed -3 to 7 DAT. Overall condition of the colonies (food stores, brood status and colony strength) were assessed –2, 4, 7, 13, 20 and 26 DAT, while detailed brood assessments were made on -2, 4, 7, 13 and 20 DAT. Colony strength and condition assessments were conducted according to the study author's assumption that the maximum number of bees per colony consisting of one super with a total of 11 combs and two bounding hive walls could theoretically be 21,600 bees. For assessments the study author further assumed that each comb side was separated into 8 equal subsections covered by a theoretical maximum number of 900 bees, assessments were conducted by counting the number of "eights" (subsections) covered by bees (assuming that each eight held 112.5 bees), and then extrapolating the number of subsections per comb to the estimated total number of bees per colony.

Detailed brood development of single brood cells was performed using the NEXTREAT™ digital image analysis tool, with brood frames (300 cells) containing eggs observed over one complete brood cycle of 21 days. Detailed cell-level brood development evaluations were made -2, 4, 7, 13 and 20 DAT; in each evaluation, digital images were taken of combs, and the content of individual cells (*i.e.*, empty, egg, young larvae [L1-L2], old larvae [L3-L5], pupae [capped cells], nectar, pollen, or dead) was color-coded by the NEXTREAT™ software. Brood termination rates were calculated based on the failure of individual eggs or larvae to develop successfully. For calculation of the brood index and brood compensation index, the color-coded images for each assessment day were then compared to the bee brood development stage expected for each assessment day (process depicted **Figure 1**).

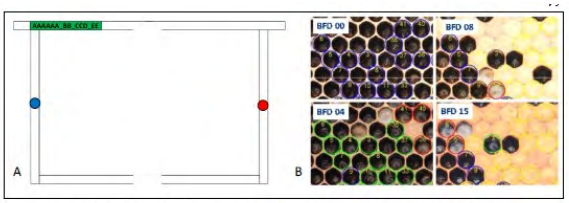


Figure 4: Detailed brood assessment with Nextreat digital image analysis tool

A) Schematic description of the initial comb labels; B) Schematic description of marking cells with the respective cell content using Nextreat tool

Table 6: Evaluation of detailed bee brood development (chronological test schedule)

Date of assessment*	Expected brood stage
BFD 0 (DAT -2)	Egg
BFD 6	Young larvae or old larvae
BFD 10	Pupae
BFD 16	Pupae
BFD 21	Empty cells or refilled after hatch

DAT = day after treatment; BFD = Brood area fixing day

Figure 1. Details on evaluation of bee brood development using NEXTREAT software (copied from registrant-submitted study report).

Test Environmental Conditions:

Ambient environmental conditions inside the tunnels (weather data for -4 to 7 DAT collected at an undescribed location at the test site; data for 8 to 26 DAT acquired at the monitoring site) and reported here as daily means: 13.2-19.1 °C and 55-85% relative humidity (RH) before application; 25.4-36.0 °C and 38.6-45.2% RH during treatments; 13.2-23.4°C and 53-75% RH during the 7-d exposure phase in the tunnels; and, 12.9-22.0°C and 53-84% RH between 8 and 26 DATS during the monitoring phase. Rainfall (>1.0 mm) was reported during the study at -4, -3, 3, 7, 8, 13, 15, 17, 18 and 22 DAT and consisted of 4, 5, 9, 5.5, 2.4, 3.4, 2.2, 2.9, 12.7 and 4.0 mm, respectively.

Photoperiod and Lighting: Natural

Nominal and Measured Concentrations:

Negative control: tap water (400 L/ha)

Test item: afidopyropen: 0.5 L/ha (50 g a.i./ha [nominal])
Reference item I – fenoxycarb: 300 g a.i./ha (nominal)
Reference item II - dimethoate: 480 g a.i./ha (nominal))

Test Plots: The test site was located in 04808 Kuhren-Burkhartshain, Saxony, Germany. For the control and afidopyropen treatments, four separate tunnels (*i.e.*, replicates), were set up within a field of fully flowering *P. tanacetifolia*; three tunnels were used for the reference item I (fenoxycarb) treatments, and a single tunnel used for the reference item II (dimethoate) treatment. Each tunnel was 18 m length x 6 m width x 2.5 m height (108 m² floor space).

Test Design: Tunnel test under semi-field conditions, with one bee hive per 108 m² tunnel. Tunnels were set up on a field of *P. tanacetifolia*, and healthy bee colonies were introduced on 1 June 2015, at BBCH development stage 63-65 (30-50% open flowers) of the crop, at four days before treatment (DAT -4). The treatment was carried out four days later during bee flight at full flowering of the crop (BBCH 65, full flowering). Bees were exposed to the water, afidopyropen or reference item (fenoxycarb or dimethoate)-treated phacelia in the tunnels for seven days. At 7 DAT, colonies were removed from the tunnels and relocated to a monitoring site approximately 5.5 km southeast. The monitoring site (Bennewitz/Altenbach, Germany) was located in a forested area with no bee attractive crops.

III. APPLICANT'S REPORTED RESULTS AND DISCUSSION

Exposure Duration: 7 d

Endpoint(s): Adult and pupal mortality, foraging activity of adult honeybees, brood strength and condition, and brood development.

Effect Concentration: ≥ 0.5 L/ha

Basis for Concentration: Nominal

Effect Concentration Type:	Test material
Basis for Effect:	Adult mortality, foraging activity, sublethal behavioral effects, colony strength and condition, brood strength.

Applicant-Provided Results:

Application Conditions & Deviations: Treatments were made using a single plot sprayer (Model PL 1, agrotop GmbH, Obertraubling, Germany) hand-held boom sprayer. Treatments to the negative control, afidopyropen and reference item tunnels were made between 10:57 am and 12:36 PM on 5 June 2015. Mean bee foraging activity prior to daytime applications was reported to be 15.3 bees/m^2 in study tunnels. At the time of applications, wind speed outside tunnels for all applications was 0.5-0.9 m/s, temperatures were 24.2-26.0 °C, and relative humidity was 38.6-40.2%. The amount of applied product (based on application volumes) deviated from the target application amount by –4.5 to 2.9% for afidopyropen applications, and -0.8 to 4.0% for the fenoxycarb and dimethoate applications.

Sublethal Behavioral Effects: Sublethal behavioral effects were observed and recorded for negative control, afidopyropen, and fenoxycarb/dimethoate tunnels for the duration of the study. According to the study authors, there were no sublethal behavioral effects observed in negative control or reference item (fenoxycarb or dimethoate) colonies at any point during the study. In afidopyropen-treated tunnels, 1-2 hours after applications on average 50 bees in each tunnel were reported to exhibit impaired locomotion, and in a few cases moribund behavior. These same sublethal effects were reported to have occurred in several bees per colony through the end of 2 DAT; additionally, over the same time span the study author reported that foraging bees exhibited uncoordinated movements on treated flowers, and fell down to the ground. No sublethal behavioral effects were reported in afidopyropen-treated tunnels from 3 DAT until the conclusion of the study (26 DAT).

Adult & Juvenile Mortality: According to the study author, there were no statistically significant differences in adult worker bee mortality between the negative control colonies and either the colonies from afidopyropen-treated tunnels or the fenoxycarb- or dimethoate-treated colonies during the pre-application and monitoring phases of the study (see **Table 1**). During the exposure phase of the study, mean adult worker bee mortality was reportedly significantly ($p<0.05$) different (*i.e.*, higher) in colonies treated with afidopyropen relative to control colonies. Apparently, beginning on the day of application following treatment (*i.e.*, 0aa DAT) and through 3 DAT, adult worker bee mortality in colonies treated with afidopyropen was significantly ($p <0.05$) higher than in negative control colonies; for example, mean worker bee mortality on 0aa DAT in afidopyropen treated colonies was 6.1x higher ($p<0.05$) than in negative control colonies.

According to the study author, during the pre-application, exposure and monitoring phases, no dead pupae were found in negative control or afidopyropen-treated colonies; therefore, the study author did not perform statistical analyses on pupal mortality data (**Table 1**). During the monitoring phase of the study, mean mortality of pupae was 13.1 dead pupae/colony/d in fenoxycarb-treated colonies, compared to no dead pupae reported in the negative control colonies during this same time period.

Table 1. Study author-reported effects on bee (*Apis mellifera*) mortality and foraging activity under semi-field conditions (tunnel test) at pre-application, in-tunnel exposure phase, and post-exposure monitoring phase for negative control, formulated afidopyropen-treated (BAS 440 00 I, 9.8% a.i.), and dimethoate or fenoxycarb -treated colonies (means ± standard deviation are reported [except for dimethoate]).

	Control	Afidopyropen	Fenoxycarb [1]	Dimethoate [2]
Mean mortality of adult worker bees (n dead bees/colony/day)				
Pre-application phase (-4 – 0 DAT) [3]	18.7 ± 9.8	19.0 ± 9.4	18.1 ± 8.6	17.8
Exposure phase (0 – 7 DAT) in the tunnels [3]	21.8 ± 10.2	45.5 ± 36.3 *	25.3 ± 12.0	133.1
Monitoring phase (8 – 26 DAT) outside the tunnels [4]	5.2 ± 5.7	5.0 ± 4.6	4.5 ± 4.6	7.6
Mean mortality of pupae (n dead pupae/colony/day) [5]				
Pre-application phase (-4 – 0 DAT)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
Exposure phase (0 – 7 DAT) in the tunnels	0.0 ± 0.0	0.0 ± 0.0	13.1 ± 23.0	0.0
Monitoring phase (8 – 26 DAT) outside the tunnels	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
Mean foraging activity (bees/m^2/colony/day [n])				
Pre-application phase (-4 – 0 DAT)	12.3 ± 3.3	12.6 ± 3.3	12.1 ± 2.8	13.2
Exposure phase (0 – 7 DAT) in the tunnels	16.8 ± 6.0	15.0 ± 5.9	16.3 ± 6.2	0.5

1) Mean value of three replicate tunnels.
2) Value represents data collected from a single tunnel, so no standard deviation is calculated.
3) Sum of dead individuals found in dead bee traps and on linen sheets in the tunnels.
4) Mean number of dead honeybees per day and colony found in dead bee traps, only.
5) Data on mean mortality of pupae were not statistically analyzed by the study author.

* = statistically significant differences ($p < 0.05$) compared to the control, Student's t-test or Welch's t-test
DAT = days after treatment

Foraging Activity: According to the study authors, there were no statistically significant differences in mean bee foraging activity between colonies from the negative control and either the afidopyropen-treated tunnels or the fenoxycarb-treated colonies at any point in the study (see **Table 1**). Applications of dimethoate resulted in a substantial reduction in mean bee foraging behavior (0.5 bees/m^2/colony/d) relative to negative control colonies (mean: 16.8 bees/m^2/colony/d) during the exposure phase of the study, but because this treatment group only contained a single tunnel, no statistical comparisons could be made.

Colony Strength: The study author did not appear to statistically analyze colony strength (*i.e.*, estimated number of bees per colony) data, but nevertheless stated that there was no indication of adverse effects from afidopyropen treatments on overall colony strength (see **Table 2**). Similarly, by the end of the study, average colony strength in colonies treated with fenoxycarb or dimethoate appeared to be roughly similar to colony strength in negative control colonies; also, fenoxycarb or dimethoate treatments did not reduce overall colony strength relative to initial (-2 DAT) levels.

Table 2. Summary of colony strength (mean number of worker bees) in negative control, afidopyropen-treated (BAS 440 00 I, 9.8% a.i.), and reference item (fenoxycarb & dimethoate) colonies at specified days after treatment (DAT). Table reproduced from applicant-submitted study report.

Assessment day	Colony strength [estimated average number of bees/colony]										
	Control			Test item			Reference item I (Fenoxycarb)			Reference item II (Dimethoate)	
	Mean[1]	± SD	%[4]	Mean[1]	± SD	%[4]	Mean[2]	± SD	%[4]	Mean[3]	%[4]
BFD 0 (DAT -2)	6834	626	-	7059	489	-	7013	325	-	7988	-
BFD 6 (DAT 4)	8803	1123	+29	9028	1391	+28	9600	1045	+37	7763	-3
BFD 9 (DAT 7)	8831	762	+29	7975	356	+12	8213	516	+17	4950	-38
BFD 15 (DAT 13)	11166	1092	+63	9731	1382	+38	10200	426	+45	8213	+3
BFD 22 (DAT 20)	10631	749	+56	9450	968	+34	9150	687	+30	7763	-3
BFD 28 (DAT 26)	13725	566	+101	11138	1504	+58	9450	338	+35	10463	+31

DAT: day after treatment; BFD: Brood area fixing day;
[1] mean of four replicates; [2] mean of three replicates
[3] one replicate (no standard deviation)
[4] relative change [%] in comparison with BFD 0 (DAT -2) calculated from the respective mean values

Colony Condition: According to the study authors, overall, treatment with afidopyropen did not result in any adverse effects on brood strength (*i.e.*, estimated brood area occupied by eggs, larvae or pupae) or food stores (*i.e.*, estimated brood area occupied by nectar and pollen) (see **Tables 3** and **4**). Both fenoxycarb and dimethoate treatments resulted in substantial reductions in brood strength during the exposure phase of the study (4 and 7 DATs), but by the conclusion of the study brood strength in the fenoxycarb- and dimethoate-treated colonies had recovered to levels that were similar to negative control colonies (see **Table 3**). Furthermore, the study author reported that food stores in reference item colonies were similar to those in control colonies throughout the study period.

Table 3. Summary of brood strength (estimated brood area per colony) in negative control, afidopyropen-treated (BAS 440 00 I, 9.8% a.i.) and reference item (fenoxycarb & dimethoate) colonies at specified days after treatment (DAT). Table reproduced from applicant-submitted study report.

Assessment day	Estimated total brood or food area per colony[1] differentiated according to the brood stages of food [cm²/colony]										
	Control			Test item			Reference item I (Fenoxycarb)			Reference item II (Dimethoate)	
	Mean[1]	± SD	%[4]	Mean[1]	± SD	%[4]	Mean[2]	± SD	%[4]	Mean[3]	%[4]
Brood stage	**Eggs**										
BFD 0 (DAT -2)	1663	338	-	1702	399	-	1478	158	-	2888	-
BFD 6 (DAT 4)	1302	220	-22	1225	506	-28	1289	225	-13	1341	-54
BFD 9 (DAT 7)	1392	133	-16	1302	351	-23	1238	473	-16	206	-93
BFD 15 (DAT 13)	2076	368	+25	2153	508	+27	1994	60	+35	1753	-39
BFD 22 (DAT 20)	1534	720	-8	2101	814	+23	1220	284	-17	2269	-21
BFD 28 (DAT 26)	2140	195	+29	1470	501	-14	1203	598	-19	1547	-46
Brood stage	**Larvae**										
BFD 0 (DAT -2)	2063	552	-	2089	526	-	1788	315	-	2063	-
BFD 6 (DAT 4)	1895	160	-8	1947	199	-7	430	284	-76	0	-100
BFD 9 (DAT 7)	1650	438	-20	1160	389	-44	447	390	-75	0	-100
BFD 15 (DAT 13)	1354	446	-34	1573	389	-25	1616	604	-10	1444	-30
BFD 22 (DAT 20)	2140	229	+4	2179	208	+4	2389	79	+34	2166	+5
BFD 28 (DAT 26)	2862	638	+39	2888	706	+38	2200	537	+23	2166	+5
Brood stage	**Pupae**										
BFD 0 (DAT -2)	3790	885	-	3765	836	-	4297	1141	-	3919	-
BFD 6 (DAT 4)	3326	271	-12	3855	456	+2	4022	627	-6	2269	-42
BFD 9 (DAT 7)	3919	326	+3	4126	326	+10	3266	215	-24	1960	-50
BFD 15 (DAT 13)	3868	927	+2	3687	893	-2	1169	834	-73	206	-95
BFD 22 (DAT 20)	5092	277	+34	4177	909	+11	4435	338	3	3197	-18
BFD 28 (DAT 26)	6214	632	+64	5518	807	+47	6360	529	48	6652	+70
Brood stage	**Entire Brood (eggs, larvae + pupae)**										
BFD 0 (DAT -2)	7516	1585	-	7555	1364	-	7563	975	-	8870	-
BFD 6 (DAT 4)	6523	195	-13	7026	599	-7	5741	636	-24	3610	-59
BFD 9 (DAT 7)	6962	450	-7	6588	949	-13	4951	516	-35	2166	-76
BFD 15 (DAT 13)	7297	614	-3	7413	1163	-2	4779	476	-37	3404	-62
BFD 22 (DAT 20)	8767	942	+17	8457	838	+12	8045	372	+6	7632	-14
BFD 28 (DAT 26)	11216	1324	+49	9875	1210	+31	9764	965	+29	10365	+17

DAT: day after treatment; BFD: Brood area fixing day;
[1] mean of four replicates; [2] mean of three replicates
[3] one replicate (no standard deviation)
[4] relative change [%] in comparison with BFD 0 (DAT -2) calculated from the respective mean values

Table 4. Summary of food stores (nectar, honey and pollen) in negative control, afidopyropen-treated (BAS 440 00 I, 9.8% a.i.) and reference item (fenoxycarb & dimethoate) colonies at specified days after treatment (DAT). Table reproduced from applicant-submitted study report.

Assessment day	Estimated total brood or food area per colony[2] differentiated according to the brood stages of food [cm²/colony]										
	Control			Test item			Reference item I (Fenoxycarb)			Reference item II (Dimethoate)	
	Mean[1]	± SD	%[4]	Mean[1]	± SD	%[4]	Mean[2]	± SD	%[4]	Mean[3]	%[4]
Food stage	**Nectar**										
BFD 0 (DAT -2)	3017	604	-	3146	381	-	2785	806	-	2682	-
BFD 6 (DAT 4)	3559	824	+18	4860	1439	+55	3009	158	+8	3300	+23
BFD 9 (DAT 7)	3997	1156	+32	5092	1714	+62	4332	676	+56	4744	+77
BFD 15 (DAT 13)	5763	1349	+91	5337	422	+70	4882	980	+75	4847	+81
BFD 22 (DAT 20)	4229	1537	+40	5131	1443	+63	5999	522	+115	6137	+129
BFD 28 (DAT 26)	7967	954	+164	6124	1765	+95	6652	389	+139	7220	+169
Food stage	**Pollen**										
BFD 0 (DAT -2)	890	411	-	619	188	-	361	136	-	206	-
BFD 6 (DAT 4)	1392	496	+57	658	293	+6	688	119	+90	413	+100
BFD 9 (DAT 7)	1392	355	+57	1083	111	+75	1100	315	+205	206	0
BFD 15 (DAT 13)	1779	764	+100	683	428	+10	1616	260	+348	825	+300
BFD 22 (DAT 20)	1934	856	+117	993	481	+60	1891	570	+424	1392	+575
BFD 28 (DAT 26)	2385	674	+168	928	759	+50	2338	465	+548	1702	+725
Food stage	**Entire food (nectar + pollen)**										
BFD 0 (DAT -2)	3906	274	-	3765	542	-	3146	852	-	2888	-
BFD 6 (DAT 4)	4951	436	+27	5518	1702	+47	3696	60	+17	3713	+29
BFD 9 (DAT 7)	5389	843	+38	6175	1708	+64	5432	487	+73	4951	+71
BFD 15 (DAT 13)	7542	808	+93	6021	806	+60	6498	917	+107	5673	+96
BFD 22 (DAT 20)	6162	1308	+58	6124	1455	+63	7890	794	+151	7529	+161
BFD 28 (DAT 26)	10352	1246	+165	7052	2483	+87	8990	298	+186	8921	+209

DAT: day after treatment; BFD: Brood area fixing day;
[1] mean of four replicates; [2] mean of three replicates
[3] one replicate (no standard deviation)
[4] relative change [%] in comparison with BFD 0 (DAT -2) calculated from the respective mean values

Brood Development Indices: According to the study author, there were no significant differences in mean brood index, brood compensation index, or brood termination rate values in colonies receiving afidopyropen treatments relative to negative control colonies (see **Table 5**). On the other hand, the mean brood index and mean brood compensation index values in colonies receiving the fenoxycarb treatments were reportedly significantly ($p<0.05$) lower at BFD2 6, 9, 15 and 22, and the mean brood termination rate in the same colonies was significantly ($p<0.05$) higher throughout the study, relative to control colonies.

Table 5. Summary of brood development indices (brood index, brood compensation index, and brood termination rate) in negative control, afidopyropen-treated (BAS 440 00 I, 9.8% a.i.) and fenoxycarb-treated colonies at specified days after treatment (DAT). Table reproduced with minor edits from applicant-submitted study report.

Assessment day	Mean brood index of initially labelled eggs [%]					
	Treatment group					
	Control		Test item		Reference item I (Fenoxycarb)	
	Mean[1]	± SD	Mean[1]	± SD	Mean[2]	± SD
BFD 6	2.7	0.3	2.7	0.7	1.1*	1.2
BFD 9	3.4	0.2	3.0	0.6	1.3*	1.6
BFD 15	3.3	0.3	3.0	0.6	1.1*	1.6
BFD 22#	4.2	0.3	3.8	0.7	1.4*	1.9

Assessment day	Mean brood compensation index of initially labelled eggs [%]					
	Treatment group					
	Control		Test item		Reference item I (Fenoxycarb)	
	Mean[1]	± SD	Mean[1]	± SD	Mean[2]	± SD
BFD 6	2.7	0.2	2.7	0.7	1.1*	1.2
BFD 9	3.4	0.2	3.0	0.5	1.3*	1.6
BFD 15	3.4	0.3	3.2	0.4	1.2*	1.6
BFD 22#	4.3	0.3	4.1	0.5	2.3*	1.3

Assessment day	Mean brood termination rate of initially labelled eggs [%]					
	Treatment group					
	Control		Test item		Reference item I (Fenoxycarb)	
	Mean[1]	± SD	Mean[1]	± SD	Mean[2]	± SD
BFD 6	13.3	5.0	19.8	13.2	66.0*	39.5
BFD 9	14.6	6.7	23.2	13.7	67.7*	39.0
BFD 15	16.5	6.6	24.3	14.4	72.8*	38.8
BFD 22#	16.9	6.9	24.8	14.7	73.0*	38.4

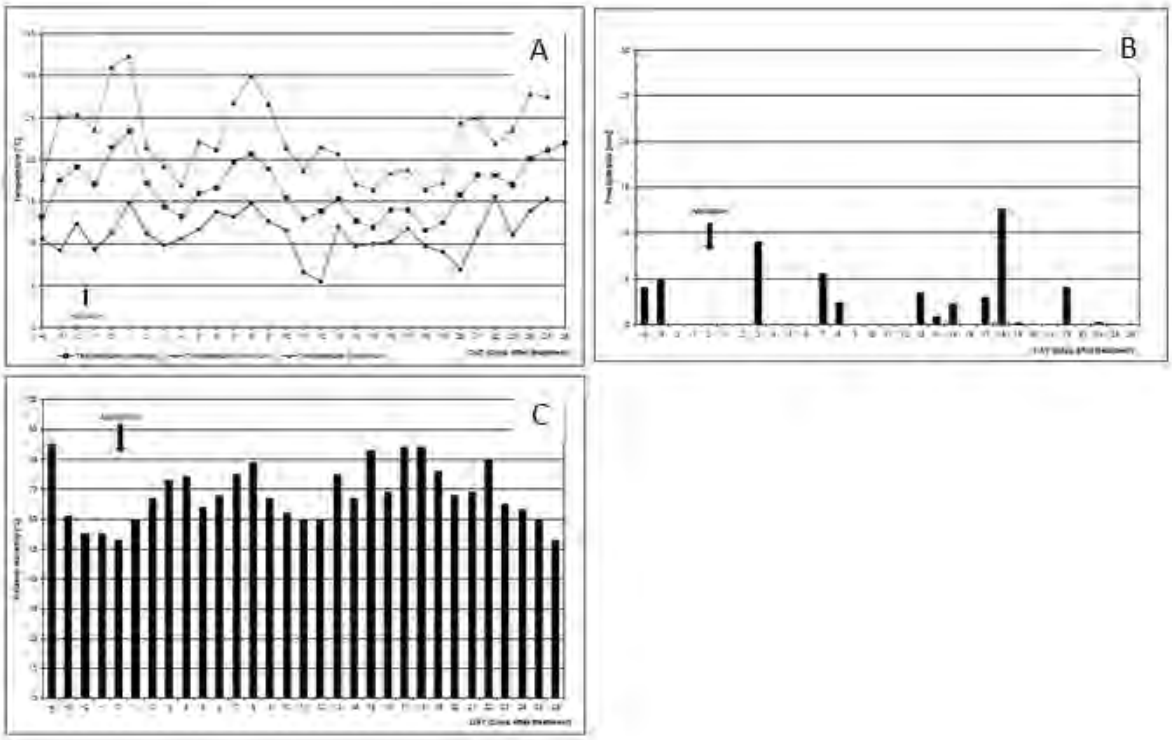
BFD: Brood area fixing day; [1] mean of four replicates; [2] mean of three replicates
* = statistically significantly different (STUDENT t-test) one-sided greater, $p<0.05$, if relevant;
# = last relevant assessment when development is expected to be completed

Residues: The study author reported that no residues of afidopyropen or its transformation product M4401007 were found in flower, leaf, nectar or pollen specimens collected at random locations in the negative control or afidopyropen tunnels before applications were made; additionally, no residues were reportedly found in specimens collected in negative control treatment tunnels following treatments. Immediately following (<4 h) applications, residues of afidopyropen and M4401007 in *Phacelia* flowers were 4.69-5.87[2] and 2.72-4.02 mg a.i./kg, respectively. Foliage residue levels were 2.41-5.62 and 2.68-5.13 mg a.i./kg, respectively, for afidopyropen and M4401007. Afidopyropen and M4401007 residues in nectar were 0.014 and 0.011 mg a.i./kg, respectively; and, residues in pollen were 0.14 and 0.05 mg a.i./kg, for afidopyropen and M4401007, respectively.

[2] Note that there is a discrepancy in the study report regarding the upper bounds of afidopyropen residues in flowers. While the text of the study report gives the range of afidopyropen floral residues as "4.69-*7.68* mg/kg," Table 17 (p. 60) of the study report gives the range of afidopyropen floral residues as "4.69-*5.87* mg/kg." This later

Weather Data: Weather data reported by the study author is summarized in **Figure 2**, and includes total daily precipitation (mm), daily mean temperature (°C), and daily mean humidity (% RH) for the study. The study author noted that during the pre-application phase of the study, mean daily temperatures were 13.2-19.1 °C, and there was 4 and 5 mm of rainfall, respectively, at -4 and -3 DAT. During the exposure phase of the study, daily minimum temperatures were below 10 °C at 3 DAT, and there was precipitation at 3 (9 mm) and 7 DATs (5.5 mm). During the monitoring phase of the study, daily minimum temperatures were below 10 °C at 11, 12, 14, 18, 19, and 20 DATs, and there was 2.4, 3.4, 2.2, 2.9, 12.7, and 4.0 mm of precipitation, respectively, at 8, 13, 15, 17, 18, and 22 DATs.

Figure 2. Summary of study author-provided data on daily temperature ('A'), precipitation ('B'), relative humidity ('C'), and cloud cover ('D').



Overall, the study author concluded that applications of BAS 440 00 I during bee flight (*i.e.*, during the daytime) resulted in some transient effects on worker bee mortality, but that there were no long-term effects on the evaluated colony parameters.

Applicant-Reported Statistics and Error Estimates

The applicant reported means and standard deviations for all endpoints, included calculated brood indices; the following endpoints were statistically analyzed by the study author: adult worker bee mortality; foraging activity; brood index; brood compensation index; and, brood termination rate. Easy

value is also given in Appendix 35 of the study report, the "Analytical Phase Report," and so was considered by the reviewer to be the correct upper bound of afidopyropen floral residues.

Assay 4.0 and ToxRat Professional (ver. 3.0 beta) were used for all of the study author's statistical analyses.

Data were apparently tested for the homogeneity of variances per the study author's descriptions of statistical methods in the study report, but it is not clear what test was used for the comparison of variances, and it's not stated whether the distribution of data were tested for normality. Pre-treatment data were statistically evaluated using a Tukey's Test, and post-treatment data were statistically evaluated using pairwise Student t-tests or Welch t-test for comparisons versus the control. All pre-application comparisons were made using two-sided tests, and all post-application comparisons were made using one-sided tests (*i.e.*, "greater" for mortality and brood termination rate, and "smaller" for foraging activity, brood index and brood compensation index).

IV. OVERALL REMARKS, ATTACHMENTS

Microsoft Excel data tables were submitted with an OECD-formatted summary by the registrant. The applicant did not include raw data on measured residues in the Excel tables, and so these data were manually extracted from the study report by the reviewer.

V. PRIMARY REVIEWER'S ANALYSIS AND CONCLUSIONS

The reviewer verified all of the applicant's calculations (where possible – see following note) and carried out statistical analyses per relevant EFED guidance for all data to confirm the applicant's results and conclusions. The study author provided only summary data for the detailed (cell-level) evaluation of brood development indices (brood index, brood compensation index, and brood termination rate), as such it was not possible for the reviewer to thoroughly verify the study author's calculations of replicate-level brood development indices. Replicate-level means for these data were extracted by the reviewer from the study report and used to confirm statistical conclusions.

Note that data on brood strength (mean number of eggs, larvae, and pupae), and food stores (mean number of cells as nectar or pollen), were expressed by the study author as colony area (*i.e.*, cm^2/colony). These colony area values were in turn estimated by the study author on the basis of the number of x/8 per frame side (number of "eights") counted as containing each type of brood or food cell, which is how the raw data on these variables were recorded by the study author. The underlying assumption was that each comb/frame side (total area of 825.1 cm^2) consisted of 8 equal parts (each 103.1 cm^2) covered by brood, food, or empty cells; the total possible comb area per colony based on these assumptions was reported by the study author to be 18,152 cm^2. The study author further explained the assumption that each frame/comb side could by covered by a maximum of 900 bees, and so each "eighth" was assumed to contain 112.5 bees. So, data were expressed and analyzed (both by the study author and the reviewer) as estimated colony area (cm^2/colony) for brood strength (mean number of eggs, larvae, and pupae) and food stores (mean number of cells as nectar or pollen); data on the mean number of worker bees were expressed and analyzed (both by the study author and the reviewer) as the mean number of bees as estimated by extrapolation from the described area to bee population density assumptions.

Adult & Juvenile Mortality: There were no statistically significant ($p < 0.05$) differences in adult worker bee mortality between afidopyropen treatments or fenoxycarb treatment groups and the negative control during the pre-application phase of the study; worker bee mortality during this phase in the single dimethoate-treated colony was similar to the negative control colonies (**Table 6**) although as

noted earlier, statistical comparisons were not possible for the dimethoate tunnel since it was not replicated. During the exposure phase, mean adult honey bee mortality was significantly ($p < 0.05$) different (*i.e.*, 209% higher) in afidopyropen-treated colonies compared to negative control tunnels; during the same phase, mean adult honey bee mortality in fenoxycarb-treated colonies was comparable to worker bee mortality in negative control tunnels. Worker bee mortality during the exposure phase in the single dimethoate tunnel (152.44 dead bees/colony/d) was higher than mean worker bee mortality in the negative control tunnels (19.39 dead bees/colony/d), but this difference could not be statistically tested. There were no statistically significant ($p < 0.05$) differences in adult worker bee mortality between afidopyropen treatment or fenoxycarb treatment groups and the negative control during the monitoring phase of the study; worker bee mortality during this phase in the single dimethoate-treated colony was similar to the negative control colonies.

Data on mean mortality of pupae were not analyzed statistically by the reviewer due to measurable mortality (per reported data) only occurring in a single treatment group at a single point in the study (*i.e.*, mean: 0.41 dead pupae/colony/d in fenoxycarb-treated colonies during the exposure phase of the study). There was no mortality of pupae reported amongst all other treatment groups at all other time points in the study (**Table 6**).

Table 6. Reviewer-calculated effects on bee (*Apis mellifera*) mortality, foraging activity, and bee brood development under semi-field conditions (tunnel test) at pre-application, in-tunnel exposure phase, and post-exposure monitoring phase for negative control, formulated afidopyropen (BAS 440 00 I; 9.8% active ingredient)-treated, and dimethoate or fenoxycarb (reference)-treated colonies (means ± standard deviation are reported [except for dimethoate]).

	Control	Afidopyropen	Fenoxycarb [1]	Dimethoate [2]
Mean mortality of adult worker bees (n dead bees/colony/day)				
Pre-application phase (-4 – 0 DAT) [3]	18.69 ± 2.46	19.00 ± 2.34	18.08 ± 2.48	17.75
Exposure phase in the tunnels (0 – 7 DAT) [3]	19.39 ± 1.92	40.44 ± 5.81†	22.44 ± 2.52	152.44
Monitoring phase outside the tunnels (8 – 26 DAT) [4]	5.16 ± 0.65	5.03 ± 0.53	4.51 ± 0.61	7.58
Mean mortality of pupae (n dead pupae/colony/day) [5]				
Pre-application phase (-4 – 0 DAT)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
Exposure phase in the tunnels (0 – 7 DAT)	0.00 ± 0.00	0.00 ± 0.00	0.41 ± 0.34	0.00
Monitoring phase outside the tunnels (8 – 26 DAT)	0.00 ± 0.00	0.00 ± 0.00	12.91 ± 3.05	0.00
Mean foraging activity (bees/m²/colony/day [n])				
Pre-application phase (-4 – 0 DAT)	12.27 ± 0.82	12.63 ± 0.81	12.06 ± 0.80	13.16
Exposure phase in the tunnels (0 – 7 DAT)	17.12 ± 0.58	13.61 ± 0.62*	17.03 ± 0.71	1.23

1) Mean value of three replicate tunnels.
2) Value represents data collected from a single tunnel, so no standard deviation is calculated; treatment group is excluded from all statistical analyses.
3) Sum of dead individuals found in dead bee traps and on linen sheets in the tunnels.
4) Mean number of dead honeybees per day and colony found in dead bee traps, only.
5) Data on mean mortality of pupae were not statistically analyzed.

* = statistically significant differences ($p < 0.05$) compared to the control, Dunnett's test

† = statistically significant differences ($p < 0.05$) compared to the control, Wilcoxon rank sum test
DAT = days after treatment

Foraging Activity: There were no statistically significant ($p < 0.05$) differences in foraging activity between afidopyropen or fenoxycarb treatment groups and the negative control during the pre-application phase of the study; foraging activity during this phase in the single dimethoate-treated colony was similar to the negative control colonies (**Table 6**). During the exposure phase of the study, relative to negative control colonies mean foraging activity was significantly ($p < 0.05$) different (*i.e.*, 21% lower) in afidopyropen-treated tunnels, but was comparable in fenoxycarb-treated tunnels; foraging activity in the single dimethoate-tunnel was 93% lower than in negative control tunnels, but this difference could not be statistically tested.

Colony Strength: The mean number of adult worker bees in afidopyropen and fenoxycarb-treated tunnels was comparable to that in the negative control tunnels throughout the study except at 26 DAT (**Table 7**). At this final assessment point, the mean number of worker bees in both afidopyropen- and fenoxycarb-treated tunnels was significantly ($p<0.05$) different (*i.e.*, lower by 19 and 32%, respectively) than the mean number of worker bees in the negative control tunnels. The mean number of adult worker bees in the single dimethoate-treated tunnel was lower (24-44%) than in control tunnels from 7 DAT through the end of the study at 26 DAT, but this difference could not be statistically tested.

Colony Condition: The mean area of brood (eggs, larvae and pupae) cells in afidopyropen-treated tunnels - as well as qualitatively in the single dimethoate tunnel - was comparable to that in control tunnels throughout the study (**Table 7**). On 7 and 13 DATs, the mean area of brood cells in fenoxycarb-treated tunnels was significantly ($p<0.05$) different (*i.e.*, 28 and 35% lower, respectively) compared to negative control tunnels. The amount of brood increased steadily over time, with on average (across treatments) a 31% increase in brood area through the single brood cycle encapsulated by the study.

The mean area of food (nectar and pollen) cells in afidopyropen- and fenoxycarb-treated tunnels - as well as qualitatively in the single dimethoate tunnel - was comparable to that in control tunnels throughout the study, except for 26 DATs (**Table 7**). On 26 DATs, the mean area of food cells in afidopyropen-treated tunnels was significantly ($p<0.05$) different (*i.e.*, 22% lower, respectively) compared to negative control tunnels. Overall, the amount of food increased over time, with on average (across treatments) a 160% increase in food area over the 26-day study period.

Table 7. Reviewer-calculated effects on honey bee (*Apis mellifera*) colony strength and condition under semi-field conditions (tunnel test) by day after treatment (DAT) for negative control, formulated afidopyropen (BAS 440 00 I; 9.8% active ingredient)-treated, and fenoxycarb or dimethoate-treated colonies (means ± standard error are reported).

	Days after Treatment (DAT)					
Treatment	**-2**	**4**	**7**	**13**	**20**	**26**
Colony Strength – Adults (est. n adult bees/colony/d)						
Control	6,838 ± 313	8,804 ± 561	8,832 ± 391	11,166 ± 546	10,632 ± 374	13,726 ± 283
Afidopyropen	7,060 ± 245	9,029 ± 696	7,875 ± 178	9,732 ± 691	9,450 ± 484	11,138 ± 752*
Fenoxycarb [1]	7,013 ± 187	9,600 ± 603	8,213 ± 298	10,200 ±246	9,150 ± 397	9,450 ± 195*
Dimethoate [2]	7,988	7,763	4,950	8,213	7,763	10,463
Colony Condition – Brood (est. cm^2/colony as eggs, larvae and pupae)						
Control	7513 ± 792	6521 ± 98	6959 ± 225	7294 ± 307	8764 ± 471	11212 ± 662

Afidopyropen	7552 ± 682	7024 ± 299	6586 ± 475	7617 ± 719	8454 ± 419	9872 ± 605
Fenoxycarb [1]	7561 ± 563	5739 ± 367	4949 ± 298 *	4777 ± 275 *	8042 ± 215	9760 ± 557
Dimethoate [2]	8867	3609	2165	3402	7629	10362
Colony Condition – Food (est. n cm^2/colony as nectar and pollen)						
Control	3905 ± 137	4949 ± 218	5387 ± 421	7539 ± 404	6160 ± 654	10684 ± 632
Afidopyropen	3763 ± 271	5516 ± 850	6173 ± 854	6173 ± 854	6289 ± 427	7049 ± 1241 *
Fenoxycarb [1]	3145 ± 492	3694 ± 34	5430 ± 281	6495 ± 529	7887 ± 458	8987 ± 172
Dimethoate [2]	2887	3712	4949	5671	7526	8918

[1)] Mean value of three replicate tunnels.

[2)] Value represents data collected from a single tunnel, so no standard error is calculated for colony strength endpoint; consequently, this treatment group is excluded from all statistical analyses.

* = statistically significant differences ($p < 0.05$) compared to the control, Dunnett's test

Brood Development Indices: The mean brood index and brood compensation index in afidopyropen-treated colonies was comparable to that in control colonies at the four assessment points in the study (**Table 8**). There were no statistical differences in mean brood termination rate between afidopyropen-treated colonies and the negative control colonies throughout the study. Compared to negative control colonies, the mean brood termination rate in afidopyropen-treated colonies was effectively 50% higher throughout the study (**Table 9**), but there was a relatively high amount of variation from the treatment mean in afidopyropen-treated colonies (mean=23.00%, variance = 161.07%).

Similarly, there were no significant differences in mean brood termination rate in fenoxycarb-treated colonies relative to the negative control colonies throughout the study, at least when a multiple comparisons test is used to compare means (see further discussion in "Reviewer Comments"). Compared to negative control colonies, the mean brood termination rate in fenoxycarb-treated colonies was effectively 358% higher throughout the study (**Table 9**), but there was a relatively high amount of variation from the treatment mean (mean= 69.86%, variance = 11,12.35%).

Table 8. Reviewer-calculated effects on honey bee (*Apis mellifera*) brood development indices under semi-field conditions (tunnel test) by day after treatment (DAT) for negative control, formulated afidopyropen (BAS 440 00 I; 9.8% active ingredient)-treated, and fenoxycarb-treated colonies (means ± standard error are reported).

	Days After Treatment (DAT)[1]			
	4	**8**	**14**	**20**
Brood Index (bi)				
Control	2.67 ± 0.12	3.41 ± 0.12	3.34 ± 0.13	4.16 ± 0.17
Afidopyropen	2.64 ± 0.37	3.02 ± 0.30	3.03 ± 0.29	3.76 ± 0.37
Fenoxycarb [2]	1.06 ± 0.68	1.29 ± 0.90	1.09 ± 0.90	1.35 ± 1.11
Brood Compensation Index (bci)				
Control	2.69 ± 0.11	3.42 ± 0.12	3.38 ± 0.13	4.29 ± 0.16
Afidopyropen	2.66 ± 0.36	3.08 ± 0.27	3.19 ± 0.19	4.09 ± 0.27
Fenoxycarb [2]	1.09 ± 0.69	1.30 ± 0.90	1.20 ± 0.92	2.28 ± 0.73
Brood Termination Rate (btr, %)				
Control	13.32 ± 2.52	14.57 ± 2.84	16.50 ± 3.29	16.93 ± 3.45
Afidopyropen	19.75 ± 6.62	23.16 ± 6.68	24.33 ± 7.19	24.75 ± 7.32
Fenoxycarb [2]	66.00 ± 22.77	67.67 ± 22.51	72.77 ± 22.42	73.00 ± 22.19

[1)] Data reported here on the basis of day after treatment, but was analyzed on the basis of brood feeding day as follows: Brood Fixation Day [BFD]6 = 4 DAT, BFD10 = 8 DAT, BFD16 = 14 DAT, and BFD22 = 20 DAT.

[2)] Mean value of three replicate tunnels; this treatment group was excluded from statistical analyses due to issues discussed in the "Reviewer's Comments" section of this document.

Table 9. Reviewer-calculated summary of mean effect (% relative to control) and variance of mean effects on honey bee (*Apis mellifera*) brood development indices under semi-field conditions (tunnel test) by day after treatment (DAT) for negative control, formulated afidopyropen (BAS 440 00 I; 9.8% active ingredient)-treated, and fenoxycarb-treated colonies.

	Mean Effect (% relative to control)				Variance (s^2)			
	4	8	14	19	4	8	14	19
Brood Index (bi)								
Control	N/A				0.06	0.06	0.07	0.12
Afidopyropen	-1.1.	-11.4	-9.3	-9.6	0.54	0.35	0.33	0.54
Fenoxycarb	-60.3	-62.2	-67.4	-67.5	1.40		2.42	3.69
Brood Compensation Index (bci)								
Control	N/A				0.05	0.06	0.06	0.11
Afidopyropen	-1.1	-9.9	-5.6	-4.7	0.53	0.29	0.14	0.30
Fenoxycarb	-59.5	-62.0	-64.5	-46.9	1.42	2.45	2.52	1.62
Brood Termination Rate (btr, %)								
Control	N/A				25.35	32.17	43.33	47.68
Afidopyropen	+48.3	+59.0	+47.5	+46.2	175.18	188.43	206.62	214.57
Fenoxycarb	+395.5	+364.4	+341.0	+331.2	1555.60	1520.33	1507.96	1477.00

Residues: Note that for analysis of afidopyropen residues in relevant matrices (*i.e.*, flowers, leaves, nectar and pollen) a single pooled sample was collected from the separate residue sampling-only afidopyropen tunnel, so no statistical analyses could be carried out on reported residue results for these data. Please reference Section III above for the study author's reported residue results. Residues were analyzed using Agilent liquid chromatography coupled with Applied Biosystems tandem mass spectroscopy (LC-MS/MS). The limit of detection (LOD) and the limit of quantification (LOQ) for both afidopyropen and M449I007 were 0.003 mg/kg and 0.01 mg/kg, respectively. Mean recovery in fortified matrices are reported to have ranged from 70 – 100%.

Reviewer's Statistical Verification:
Statistical analyses confirmed using R (ver. 3.2.5)[3] statistical software, and the multicomp[4] analysis package. The reviewer relied on the Shapiro-Wilk's test and Bartlett's test to evaluate whether data were normally distributed or homoscedastic, respectively. ANOVA and Dunnett's multiple means test were used to test for statistical differences amongst means for data that met assumptions for parametric tests (*i.e.,* data were approximately normally distributed and had homogenous variances), and Kruskal-Wallis and Wilcoxon Rank Sum test were used for non-parametric comparisons. One-sided tests were used for all hypothesis-based testing; $\alpha = 0.05$ for all mean comparison tests, and $\alpha = 0.01$ for all assumptions testing.

See **Appendix I** for summary statistics and diagnostic tests (*i.e.,* goodness-of-fit and equivalent variances tests) for all data described in this data evaluation report.

[3] R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/.
[4] Hothorn T, F Bretz and P Westfall. 2008. Simultaneous inference in general parametric models. Biometric Journal 50: 346-363.

Based on statistically significant adverse effects on adult worker honeybee mortality and foraging activity, the no-observed adverse effect level (NOAEL) across the various measurement endpoints for afidopyropen is <50 g a.i./ha under the conditions tested for this treatment.

Reviewer's Comments:
The reviewer's overall results and conclusions agreed with those of the study author, and in spite of some differences regarding approaches towards statistically analyzing the study data, the reviewer and the study author agreed on the statistical significance of treatment responses for particular endpoints. The study author did not statistically analyze colony strength or condition data, so comparisons between the reviewer's and study author's conclusions for these endpoints are not possible.

There are minor discrepancies between the study author's and reviewer's calculated treatment means for the exposure phase, this discrepancy appears to be due to a calculation difference with respect to the multiple assessments made on the day of applications (*i.e.*, 0 DAT) following applications. The study author took an average of the 0 DAT after application assessments, and then used this 0 DAT mean for subsequent calculations of the exposure phase treatment mean. The reviewer simply included the multiple 0 DAT after application assessments in the calculation of exposure phase treatment means, generally resulting in a slightly different mean value than the study author.

In terms of statistical approaches, the study author claimed in the study report that data were tested to see whether they met assumptions of parametric tests, and the statistical tests used by the author are all parametric tests. However, the reviewer's analysis indicated that several of the datasets analyzed in this manner by the study author did not meet assumptions for parametric tests, and should have been analyzed using non-parametric tests. Additionally, the study author relied on pairwise t-tests for mean comparisons, which given that there were multiple treatments to be tested was not the most appropriate choice (*i.e.*, multiple comparisons tests would have been more appropriate). Ultimately, the study author's approach to statistically analyzing the datasets resulted in the same overall conclusions as the reviewer's, with the exception of the detailed brood development indices data. Using a combination of pairwise student t-tests and Welch's t-tests (which are more appropriate when treatment mean variances differ, the study author concluded that the brood index, brood compensation index, and brood termination rate were significantly ($p<0.05$) different from the control at each of the time points assessed. The reviewer tried to replicate these results, even using the same Welch's t-tests used by the study author, and was unable to conclude that fenoxycarb treatment means were significantly ($p<0.05$) different from the negative control means.

Data provided in the study report indicate that the average time to make applications to each tunnel was 2 minutes per tunnel. Given the described application protocols in the study report, it's difficult to understand how applications could have been made to each of the tunnels in such a short timeframe.

The study author noted that during the pre-application phase of the study mean daily temperatures were 13.2-19.1 °C, and there was 4 and 5 mm of rainfall, respectively, at -4 and -3 DAT. OECD Guidance Document No. 75 notes that daytime temperatures below 15 °C may inhibit honeybee foraging activity. Additionally, during the exposure phase of the study minimum temperatures were below 10 °C at 3 DAT, and there was precipitation at 3 (9 mm) and 7 DATs (5.5 mm). While these adverse environmental conditions would have theoretically affected all treatment groups equally, nevertheless they result in

some uncertainty regarding the degree of foraging activity of colonies at the time of applications, and during the exposure phase of the study.

Study results indicate that the primary reference item (fenoxycarb) resulted in the following significant ($p < 0.05$) adverse effect relative to control colonies: a 32% reduction in the mean number of worker bees at 26 DAT, and 28-35% reduction in brood strength 7 and 13 DATs. As previously discussed, fenoxycarb treatments also appeared to adversely affect brood development, but this effect relative to the negative control could not be detected statistically due to very high amounts of variation. Specifically (with brood development indices as an example), the mean brood index and brood compensation index are much higher in tunnel #3 (mean brood index= 2.96, mean brood compensation index = 3.06) compared to the other two tunnels (mean brood index = 0.32, mean brood compensation index= 0.67), and the mean brood termination rate is much lower in tunnel #3 (mean = 25.75%) compared to the other two tunnels (mean = 91.91%).

Data from the single dimethoate-treated tunnel appeared to also show adverse treatment effects on honeybee colonies, but as this treatment was not replicated, it could not be included in statistical analyses. Collectively, these responses due to fenoxycarb and dimethoate treatments provide evidence that honeybee colonies in this study were exposed to test materials, and that to some degree the test system was able to detect treatment effects associated with both of the reference toxicants; however, the high variability limited the extent to which these effects could be statistically differentiated. The reviewer believes that as a result there is some uncertainty as to how effectively honeybee colonies in this study were exposed to reference item (fenoxycarb and dimethoate) treatments applied as part of the study and by extension also some uncertainty regarding exposure of colonies to afidopyropen treatments.

As part of the study, afidopyropen residues following applications were measured in a separate tunnel (n = 1), with measured residue levels of 7.68 and 5.62 mg a.i./kg tissue, respectively, in phacelia flowers and foliage; however, residues were only measured in a single separate tunnel, and not in the four treated tunnels in which effects were assessed, and so while measured residues do provide some evidence of exposure from applications, the data does not confirm that all four treated tunnels were exposed to a roughly similar concentrations of the test item.

Reviewer's Conclusions:
The semi-field (tunnel) bee brood study was initiated in June 2015 with the afidopyropen formulated end-use product BAS 440 00 I (VERSYS™, 9.8% active ingredient) applied to phacelia at full bloom during active bee foraging (*i.e.*, daytime). Bee colonies in the negative control, reference item (fenoxycarb: 300 g a.i./ha nominal & dimethoate: 480 g a.i./ha nominal), and 50 g a.i./ha BAS 440 00 I (0.04 lbs a.i./A) treatments were assessed at multiple time points; treatment rates were not confirmed analytically. The exposure phase was seven days (0 – 7 DAT), and the post-exposure monitoring phase was 26 days for all endpoints.

There were no statistically significant ($p < 0.05$) differences in adult worker bee mortality between afidopyropen-treated groups and the negative control during the pre-application or monitoring phases of the study; but during the exposure phase, mean adult honey bee mortality in afidopyropen-treated colonies was significantly ($p<0.05$) different (209% higher) compared to negative control tunnels. This increase in adult mortality was largely due to adverse treatment effects on the day of application

through 3 DAT, after which worker bee mortality in afidopyropen-treated tunnels was similar to that in control tunnels. There was no mortality of pupae reported in any of the afidopyropen-treated colonies at any point in the study. There were no statistically significant ($p < 0.05$) differences in foraging activity between afidopyropen-treated colonies and the negative control during the pre-application phase of the study, but during the exposure phase of the study, relative to negative control colonies mean foraging activity in afidopyropen-treated tunnels was significantly ($p<0.05$) different (21% lower). Again, this adverse effect was largely due to reduced foraging activity on 0 DAT, and for the remainder of the exposure phase, foraging activity in afidopyropen-treated tunnels was similar to that in control tunnels. The mean number of adult worker bees in afidopyropen-treated colonies was comparable to that in control tunnels throughout the study except at 26 DAT, when the mean number of worker bees in afidopyropen-treated colonies was significantly ($p<0.05$) different (19% lower) than the mean number of worker bees in the negative control tunnels. There were no significant differences in brood strength (*i.e*., amount of eggs, larvae or pupae) or food stores (*i.e*., amount of nectar or pollen) in afidopyropen-treated colonies relative to the negative control at any point in the study. Similarly, there were no significant differences in the mean brood index, brood compensation index, or brood termination rate relative to the negative control at any point in the study.

There were adverse weather conditions during the pre-application period (*i.e*., daily temperatures < 14 °C and rainfall), and 3-7 DAT (9 mm of total rainfall). There was also >5 mm rainfall periodically throughout the monitoring phase of the study. Additionally, because nominal treatment levels of afidopyropen, fenoxycarb or dimethoate were not verified analytically, there is some uncertainty regarding actual exposure levels. Measured afidopyropen residue levels indicate though that colonies were exposed to the afidopyropen treatments; however, study data from fenoxycarb-treated colonies were highly variable, and so there is additional uncertainty as to how consistent applications of the afidopyropen and reference items were across tunnels especially given that treatments for each tunnel were completed in roughly 2 minutes per tunnel.

The study was generally consistent with OECD Guidance Document 75, and indicates that honey bee colonies treated with formulated afidopyropen at 50 g a.i./ha (0.04 lbs a.i./A) during active bee flight (*i.e*., in the daytime) exhibited statistically significant ($p<0.05$) adverse effects on adult worker bee mortality (209% higher), foraging activity (21% lower), and colony strength (19% lower at 26 DAT). Overall, adverse treatment effects occurred primarily in the first several days of the exposure phase of the study, after which by almost all measures afidopyropen-treated colonies were roughly similar to negative control colonies. Based on this study and the noted statistically significant effects, the NOAEL is <50 g a.i./ha for applications during active bee flight.

EPA Classification: Supplemental (should only be used qualitatively)
PMRA Classification: Reliable with restrictions

APPENDIX I. Output of Statistics Verified by the Reviewer

Adult Honeybee Mortality (no. dead bees/colony/d)

```
Call: lm(formula = value ~ trtmnt + phase, data = z)

Residuals:
    Min      1Q  Median      3Q     Max
-28.514  -6.388  -1.084   2.916 117.486

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  25.6309     1.7033  15.048  < 2e-16 ***
trtmntref a   0.3984     1.8732   0.213 0.831681
trtmnttest    5.8828     1.7342   3.392 0.000773 ***
phasemon    -22.9458     1.6927 -13.556  < 2e-16 ***
phasepre     -9.2424     2.5137  -3.677 0.000274 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 13.87 on 347 degrees of freedom
Multiple R-squared:  0.3721,     Adjusted R-squared:  0.3648
F-statistic:  51.4 on 4 and 347 DF,  p-value: < 2.2e-16

Shapiro-Wilk normality test
W = 0.69631, p-value < 2.2e-16

Bartlett test of homogeneity of variances ~ trtmnt
Bartlett's K-squared = 105.06, df = 2, p-value < 2.2e-16

Bartlett test of homogeneity of variances ~ phase
Bartlett's K-squared = 354.39, df = 2, p-value < 2.2e-16

Pre-application Phase
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 0.061226, df = 2, p-value = 0.9699

Exposure Phase
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 10.761, df = 2, p-value = 0.004607

Pairwise comparisons using Wilcoxon rank sum test

      cont   ref a
ref a 0.3774 -
test  0.0051 0.0593

Monitoring Phase
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 0.73605, df = 2, p-value = 0.6921
```

Foraging Activity (bees/m²/d)

```
Call: lm(formula = value ~ trtmnt + phase, data = z)

Residuals:
     Min       1Q   Median       3Q      Max
-11.7296  -2.8454   0.3775   2.9940  10.8875

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  16.8454     0.5374  31.349  < 2e-16 ***
trtmntref a  -0.1158     0.7859  -0.147 0.883001
trtmnttest   -2.7329     0.7276  -3.756 0.000222 ***
phasepre     -3.4786     0.7756  -4.485 1.18e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 4.602 on 216 degrees of freedom
```

```
Multiple R-squared:  0.1478,     Adjusted R-squared:  0.1359
F-statistic: 12.48 on 3 and 216 DF,  p-value: 1.471e-07

Shapiro-Wilk normality test
W = 0.98956, p-value = 0.1117

Bartlett test of homogeneity of variances ~ trtmnt
Bartlett's K-squared = 0.27923, df = 2, p-value = 0.8697

Bartlett test of homogeneity of variances ~ phase
Bartlett's K-squared = 14.043, df = 1, p-value = 0.0001786
```

Pre-application Phase

```
            Df Sum Sq Mean Sq F value Pr(>F)
group        2    2.3   1.175   0.119  0.888
Residuals   41  404.4   9.864
```

Exposure Phase

```
             Df Sum Sq Mean Sq F value   Pr(>F)
group         2    489  244.71   10.47 5.12e-05 ***
Residuals   173   4045   23.38
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Multiple Comparisons of Means: Dunnett Contrasts
Linear Hypotheses:
                  Estimate Std. Error t value Pr(>|t|)
ref a - cont == 0 -0.09089    0.92324  -0.098 0.993069
test - cont == 0  -3.50469    0.85476  -4.100 0.000126 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Adjusted p values reported -- single-step method)
```

Colony Strength (no. adult bees/colony/d)

```
Call: lm(formula = value ~ trtmnt + dat, data = z)

Residuals:
     Min       1Q   Median       3Q      Max
-2079.99  -681.16    -1.07   663.19  2258.98

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   7600.5      373.8  20.332  < 2e-16 ***
trtmntref a  -1061.0      340.1  -3.119  0.00282 **
trtmnttest    -951.5      314.9  -3.022  0.00374 **
dat4          2137.5      465.2   4.595 2.38e-05 ***
dat7          1349.9      465.2   2.902  0.00523 **
dat13         3415.8      465.2   7.343 7.76e-10 ***
dat20         2832.8      465.2   6.090 9.68e-08 ***
dat26         4653.5      465.2  10.004 3.05e-14 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1091 on 58 degrees of freedom
  (56 observations deleted due to missingness)
Multiple R-squared:  0.6998,     Adjusted R-squared:  0.6635
F-statistic: 19.31 on 7 and 58 DF,  p-value: 4.699e-13

Shapiro-Wilk normality test
W = 0.98534, p-value = 0.6278

Bartlett test of homogeneity of variances ~ trtmnt
Bartlett's K-squared = 8.3886, df = 2, p-value = 0.01508

Bartlett test of homogeneity of variances ~ dat
Bartlett's K-squared = 22.516, df = 5, p-value = 0.0004176
```

-2 DAT

```
            Df  Sum Sq Mean Sq F value Pr(>F)
```

```
group        2  110587   55293    0.21  0.815
Residuals    8 2103516  262940
56 observations deleted due to missingness

4 DAT
            Df   Sum Sq Mean Sq F value Pr(>F)
group        2  1123022  561511   0.382  0.695
Residuals    8 11773079 1471635
56 observations deleted due to missingness

7 DAT
            Df  Sum Sq Mean Sq F value Pr(>F)
group        2 1872026  936013   2.727  0.125
Residuals    8 2745844  343231
56 observations deleted due to missingness

13 DAT
            Df  Sum Sq Mean Sq F value Pr(>F)
group        2 4248652 2124326   1.756  0.233
Residuals    8 9675708 1209464
56 observations deleted due to missingness

20 DAT
            Df  Sum Sq Mean Sq F value Pr(>F)
group        2 4522321 2261161   3.326 0.0889
Residuals    8 5438082  679760

26 DAT
            Df   Sum Sq  Mean Sq F value  Pr(>F)
group        2 32784157 16392078   16.45 0.00146 **
Residuals    8  7973438   996680
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
56 observations deleted due to missingness

Multiple Comparisons of Means: Dunnett Contrasts
Linear Hypotheses:
                  Estimate Std. Error t value Pr(>|t|)
ref a - cont == 0  -4275.2      762.5  -5.607 0.000945 ***
test - cont == 0   -2587.5      705.9  -3.665 0.011646 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Adjusted p values reported -- single-step method)

-------------------------------------------------------------------------------
Colony Condition - Brood (no. cells/colony/d as brood)
Call: lm(formula = value ~ trtmnt + dat, data = z)

Residuals:
    Min      1Q  Median      3Q     Max
-2763.5  -837.4  -170.1   607.0  3343.9

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  6779.10     344.37  19.686  < 2e-16 ***
trtmntref a -1239.35     427.26  -2.901  0.00515 **
trtmnttest   -193.31     395.57  -0.489  0.62678
dat           111.60      17.72   6.296 3.49e-08 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1370 on 62 degrees of freedom
Multiple R-squared:  0.4414,     Adjusted R-squared:  0.4144
F-statistic: 16.33 on 3 and 62 DF,  p-value: 6.233e-08

Shapiro-Wilk normality test
W = 0.97025, p-value = 0.1135

Bartlett test of homogeneity of variances ~ trtmnt
```

```
Bartlett's K-squared = 1.7308, df = 2, p-value = 0.4209

Bartlett test of homogeneity of variances ~ dat
Bartlett's K-squared = 8.3871, df = 5, p-value = 0.1362

-2 DAT
            Df   Sum Sq Mean Sq F value Pr(>F)
group        2     4691    2345   0.001  0.999
Residuals    8 15004802 1875600

4 DAT
            Df  Sum Sq Mean Sq F value Pr(>F)
group        2 2834100 1417050    5.67 0.0293 *
Residuals    8 1999474  249934
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Multiple Comparisons of Means: Dunnett Contrasts
Linear Hypotheses:
                  Estimate Std. Error t value Pr(>|t|)
ref a - cont == 0   -781.8      381.8  -2.048    0.130
test - cont == 0     502.6      353.5   1.422    0.317
(Adjusted p values reported -- single-step method)

7 DAT
            Df  Sum Sq Mean Sq F value Pr(>F)
group        2 7534884 3767442    7.85  0.013 *
Residuals    8 3839282  479910
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
Multiple Comparisons of Means: Dunnett Contrasts

Linear Hypotheses:
                  Estimate Std. Error t value Pr(>|t|)
ref a - cont == 0  -2010.4      529.1  -3.800  0.00963 **
test - cont == 0    -373.7      489.9  -0.763  0.68147
---
 (Adjusted p values reported -- single-step method)

13 DAT
            Df   Sum Sq Mean Sq F value Pr(>F)
group        2 15860204 7930102   8.147 0.0118 *
Residuals    8  7787297  973412
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
Multiple Comparisons of Means: Dunnett Contrasts

Linear Hypotheses:
                  Estimate Std. Error t value Pr(>|t|)
ref a - cont == 0  -2517.4      753.5  -3.341   0.0186 *
test - cont == 0     322.2      697.6   0.462   0.8624
---
 (Adjusted p values reported -- single-step method)

20 DAT
            Df  Sum Sq Mean Sq F value Pr(>F)
group        2  892887  446444   0.708  0.521
Residuals    8 5043750  630469

26 DAT
            Df   Sum Sq Mean Sq F value Pr(>F)
group        2  4926502 2463251   1.712  0.241
Residuals    8 11513639 1439205

-------------------------------------------------------------------------------------------
Colony Condition - Food (no. cells/colony/d as food)
Call: lm(formula = value ~ trtmnt + dat, data = z)

Residuals:
```

```
    Min      1Q  Median      3Q     Max
-3935.9  -820.7   -48.6   749.3  3203.9

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  4546.12     348.96  13.028  < 2e-16 ***
trtmntref a  -497.60     432.96  -1.149    0.255
trtmnttest   -618.60     400.84  -1.543    0.128
dat           166.87      17.96   9.291 2.37e-13 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1389 on 62 degrees of freedom
Multiple R-squared:  0.5892,     Adjusted R-squared:  0.5694
F-statistic: 29.65 on 3 and 62 DF,  p-value: 5.184e-12

Shapiro-Wilk normality test
W = 0.97555, p-value = 0.2169

Bartlett test of homogeneity of variances ~ trtmnt
Bartlett's K-squared = 2.239, df = 2, p-value = 0.3264

Bartlett test of homogeneity of variances ~ dat
Bartlett's K-squared = 17.296, df = 5, p-value = 0.003972

-2 DAT
            DfSum Sq Mean Sq F value Pr(>F)
group        2 1077396  538698   1.684  0.245
Residuals    8 2558414  319802

4 DAT
            Df  Sum Sq Mean Sq F value Pr(>F)
group        2 5803445 2901722   2.508  0.143
Residuals    8 9254847 1156856

7 DAT
             Df   Sum Sq Mean Sq F value Pr(>F)
group        2  1503466  751733    0.53  0.608
Residuals    8 11348216 1418527

13 DAT
    Df  Sum Sq Mean Sq F value Pr(>F)
group        2 3508194 1754097    2.41  0.152
Residuals    8 5821705  727713

20 DAT
      Df   Sum Sq Mean Sq F value Pr(>F)
group        2  6656250 3328125   2.091  0.186
Residuals    8 12733608 1591701

26 DAT
             Df   Sum Sq  Mean Sq F value Pr(>F)
group        2 26447476 13223738   4.512 0.0488 *
Residuals    8 23444048  2930506
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Multiple Comparisons of Means: Dunnett Contrasts
Linear Hypotheses:
                  Estimate Std. Error t value Pr(>|t|)
ref a - cont == 0    -1697       1308  -1.298   0.3735
test - cont == 0     -3634       1210  -3.002   0.0308 *
---
 (Adjusted p values reported -- single-step method)
```

Brood Index (bi)

```
Call: lm(formula = value ~ trtmnt + dat, data = z)
```

```
Residuals:
    Min      1Q  Median      3Q     Max
-3875.3 -1607.8  -732.7  1758.8  5042.8

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  2273.06     371.89   6.112 1.10e-08 ***
trtmntref a  -248.80     461.40  -0.539    0.591
trtmnttest   -309.30     427.18  -0.724    0.470
dat            83.43      19.14   4.359 2.66e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 2093 on 128 degrees of freedom
  (4680 observations deleted due to missingness)
Multiple R-squared:  0.1327,     Adjusted R-squared:  0.1123
F-statistic: 6.526 on 3 and 128 DF,  p-value: 0.0003837

Shapiro-Wilk normality test
W = 0.94922, p-value = 8.692e-05

Bartlett test of homogeneity of variances ~ trtmnt
Bartlett's K-squared = 9.6542, df = 2, p-value = 0.00801

Bartlett test of homogeneity of variances ~ bfd
Bartlett's K-squared = 1.9177, df = 3, p-value = 0.5897

BFD 6 (4 DAT)
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 3.3864, df = 2, p-value = 0.1839

Welch Two Sample t-test
t = 0.070942, df = 3.6465, p-value = 0.9472

Welch Two Sample t-test
t = 2.3223, df = 2.1276, p-value = 0.1383

BFD 10 (8 DAT)
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 4.7451, df = 2, p-value = 0.09324

Welch Two Sample t-test
t = 1.1969, df = 3.9425, p-value = 0.2983

Welch Two Sample t-test
t = 2.3368, df = 2.0706, p-value = 0.1402

BFD 16 (14 DAT)
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 4.5758, df = 2, p-value = 0.1015

Welch Two Sample t-test
t = 0.99025, df = 4.2119, p-value = 0.3755

Welch Two Sample t-test
t = 2.4805, df = 2.0864, p-value = 0.1261

BFD 22 (20 DAT)
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 4.5758, df = 2, p-value = 0.1015

Welch Two Sample t-test
t = 0.96931, df = 4.2484, p-value = 0.3843

Welch Two Sample t-test
t = 2.4987, df = 2.0957, p-value = 0.124
```

Brood Compensation Index (bci)

```
Call: lm(formula = value ~ trtmnt + dat, data = z)

Residuals:
    Min      1Q  Median      3Q     Max
-3875.3 -1607.8  -732.7  1758.8  5042.8

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  2273.06     371.89   6.112 1.10e-08 ***
trtmntref a  -248.80     461.40  -0.539    0.591
trtmnttest   -309.30     427.18  -0.724    0.470
dat            83.43      19.14   4.359 2.66e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 2093 on 128 degrees of freedom
  (4680 observations deleted due to missingness)
Multiple R-squared:  0.1327,     Adjusted R-squared:  0.1123
F-statistic: 6.526 on 3 and 128 DF,  p-value: 0.0003837

Shapiro-Wilk normality test
W = 0.94922, p-value = 8.692e-05

Bartlett test of homogeneity of variances ~ trtmnt
Bartlett's K-squared = 7.7784, df = 2, p-value = 0.02046

Bartlett test of homogeneity of variances ~ bfd
Bartlett's K-squared = 0.4788, df = 3, p-value = 0.9235

BFD 6 (4 DAT)
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 3.3864, df = 2, p-value = 0.1839

Welch Two Sample t-test
t = 0.085827, df = 3.5421, p-value = 0.9362

Welch Two Sample t-test
t = 2.3059, df = 2.1016, p-value = 0.1414

BFD 10 (8 DAT)
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 4.5967, df = 2, p-value = 0.1004

Welch Two Sample t-test
t = 1.1775, df = 4.1331, p-value = 0.3023

Welch Two Sample t-test
t = 2.3351, df = 2.0691, p-value = 0.1404

BFD 16 (14 DAT)
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 4.5967, df = 2, p-value = 0.1004

Welch Two Sample t-test
t = 0.79653, df = 5.3227, p-value = 0.4598

Welch Two Sample t-test
t = 2.3516, df = 2.0785, p-value = 0.1383

BFD 22(20 DAT)
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 4.3485, df = 2, p-value = 0.1137

Welch Two Sample t-test
t = 0.65362, df = 4.9195, p-value = 0.5427

Welch Two Sample t-test
t = 2.6754, df = 2.2002, p-value = 0.1049
```

```
--------------------------------------------------------------------------------
Brood Termination Rate (btr, %)
Call: lm(formula = value ~ trtmnt + dat, data = z)

Residuals:
    Min      1Q  Median      3Q     Max
-3875.3 -1607.8  -732.7  1758.8  5042.8

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)  2273.06     371.89   6.112 1.10e-08 ***
trtmntref a  -248.80     461.40  -0.539    0.591
trtmnttest   -309.30     427.18  -0.724    0.470
dat            83.43      19.14   4.359 2.66e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 2093 on 128 degrees of freedom
  (4680 observations deleted due to missingness)
Multiple R-squared:  0.1327,     Adjusted R-squared:  0.1123
F-statistic: 6.526 on 3 and 128 DF,  p-value: 0.0003837

Shapiro-Wilk normality test
W = 0.94922, p-value = 8.692e-05

Bartlett test of homogeneity of variances ~ trtmnt
Bartlett's K-squared = 35.798, df = 2, p-value = 1.685e-08

Bartlett test of homogeneity of variances ~ bfd
Bartlett's K-squared = 0.03002, df = 3, p-value = 0.9986

BFD 6 (4 DAT)
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 5.1439, df = 2, p-value = 0.07638

Welch Two Sample t-test ~ test item
t = -0.90744, df = 3.8504, p-value = 0.4174

Welch Two Sample t-test ~ reference item
t = -2.2992, df = 2.049, p-value = 0.1452

BFD 10 (8 DAT)
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 4.8939, df = 2, p-value = 0.08656

Welch Two Sample t-test ~ test item
t = -1.1567, df = 3.9953, p-value = 0.3118

Welch Two Sample t-test ~ reference item
t = -2.3402, df = 2.0636, p-value = 0.1402

BFD 16 (14 DAT)
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 4.5758, df = 2, p-value = 0.1015

Welch Two Sample t-test ~ test item
t = -0.99084, df = 4.2052, p-value = 0.3753

Welch Two Sample t-test ~ reference item
t = -2.4831, df = 2.0865, p-value = 0.1259

BFD 22(20 DAT)
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 4.5758, df = 2, p-value = 0.1015

Welch Two Sample t-test ~ test item
t = -0.96639, df = 4.2706, p-value = 0.3853

Welch Two Sample t-test ~ reference item
```

```
t = -2.4971, df = 2.0972, p-value = 0.1241
```